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MONOGRAPH

PONATINIB

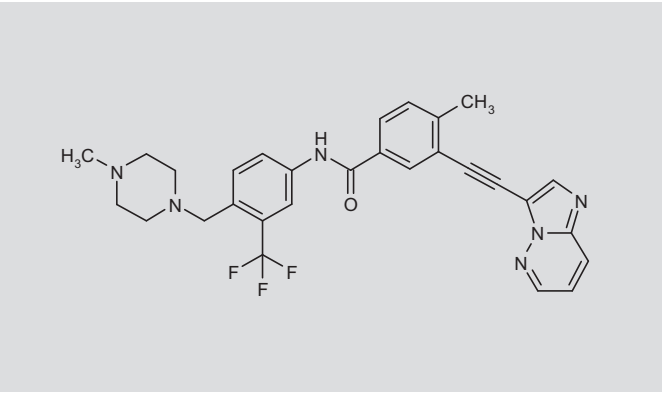
Prop INN; USAN

Multitargeted Kinase Inhibitor
Pan-BCR-ABL Inhibitor
Oncolytic

AP-24534

3-[2-(Imidazo[1,2-*b*]pyridazin-3-yl)ethynyl]-4-methyl-*N*-[4-(4-methylpiperazin-1-ylmethyl)-3-(trifluoromethyl)phenyl]benzamide

InChI: 1S/C29H27F3N6O/c1-20-5-6-22(16-21(20)8-10-25-18-33-27-4-3-11-34-38(25)27)28(39)35-24-9-7-23(26(17-24)29(30,31)32)19-37-14-12-36(2)13-15-37/h3-7,9,11,16-18H,12-15,19H2,1-2H3,(H,35,39)



$C_{29}H_{27}F_3N_6O$
Mol wt: 532.5595
EN: 440498

SUMMARY

Ponatinib (AP-24534) is a third-generation tyrosine kinase inhibitor (TKI) that addresses the clinically concerning BCR-ABL^{T315I} mutation seen in chronic myeloid leukemia (CML). This drug was originally developed as AP-24534, a "pan-BCR-ABL" inhibitor, exhibiting inhibitory effects on all currently known mutations of the BCR-ABL fusion protein. These mutations are estimated to be responsible for 40-50% of treatment failures associated with currently available TKIs. One of the most common of these mutations is the substitution of the amino acid isoleucine for the threonine at position 315 (T315I). No currently available TKI effectively inhibits this mutation, which results in resistance and subsequent treatment failure. Early data from preclinical, phase I and phase II clinical studies suggest superior response rates and manageable toxicity, indicating that ponatinib is a promising new agent for the treatment of drug-resistant CML. Herein, we will review the available literature regarding ponatinib and its use in CML patients harboring the T315I mutation.

SYNTHESIS*

Ponatinib can be prepared by two related ways:

Bromination of 1-methyl-4-nitro-2-(trifluoromethyl)benzene (I) with NBS in the presence of AIBN in refluxing CCl_4 gives the corresponding bromomethyl derivative (II), which is condensed with 1-methylpiperazine (III) by means of Et_3N in CH_2Cl_2 to afford 1-methyl-4-[4-nitro-2-(trifluoromethyl)benzyl]piperazine (IV). Reduction of compound (IV) with $Na_2S_2O_4$ in refluxing acetone/H_2O yields the aniline (V), which by coupling with 3-iodo-4-methylbenzoyl chloride (VI) (prepared by chlorinating acid [VII] with $SOCl_2$ at reflux) in the presence of DIEA and DMAP in THF provides the corresponding amide (VIII). Finally, the iodobenzene derivative (VIII) is submitted to Sonogashira coupling with 3-ethynylimidazo[1,2-*b*]pyridazine (IX) by means of CuI, $Pd(PPh_3)_4$ and DIEA in DMF (1, 2). Scheme 1.

Intermediate (IX) is prepared by Sonogashira coupling of 3-bromoimidazo[1,2-*b*]pyridazine (X) with trimethylsilyl acetylene (XI) in the presence of CuI, $PdCl_2(PPh_3)_4$ and DCHA in acetonitrile at 80 °C (1) or CuI, $Pd(PPh_3)_4$ and DIEA in DMF (2) to give 3-[(trimethylsilyl)ethynyl]imidazo[1,2-*b*]pyridazine (XII), which is deprotected by cleaving the trimethylsilyl moiety by means of TBAF in THF (1, 2). Scheme 1.

In a related way, Sonogashira coupling of 3-iodo-4-methylbenzoic acid (VII) with 3-ethynylimidazo[1,2-*b*]pyridazine (IX) in the presence of CuI, $Pd(PPh_3)_4$ and either Et_3N in EtOAc or DIEA in DMF affords 3-(imidazo[1,2-*b*]pyridazin-3-ylethynyl)-4-methylbenzoic acid (XIII). Finally, carboxylic acid (XIII) is chlorinated with $(COCl)_2$ in the presence of NMM in CH_2Cl_2, followed by amidation with the aniline derivative (V) by means of DMAP at reflux (2). Scheme 2.

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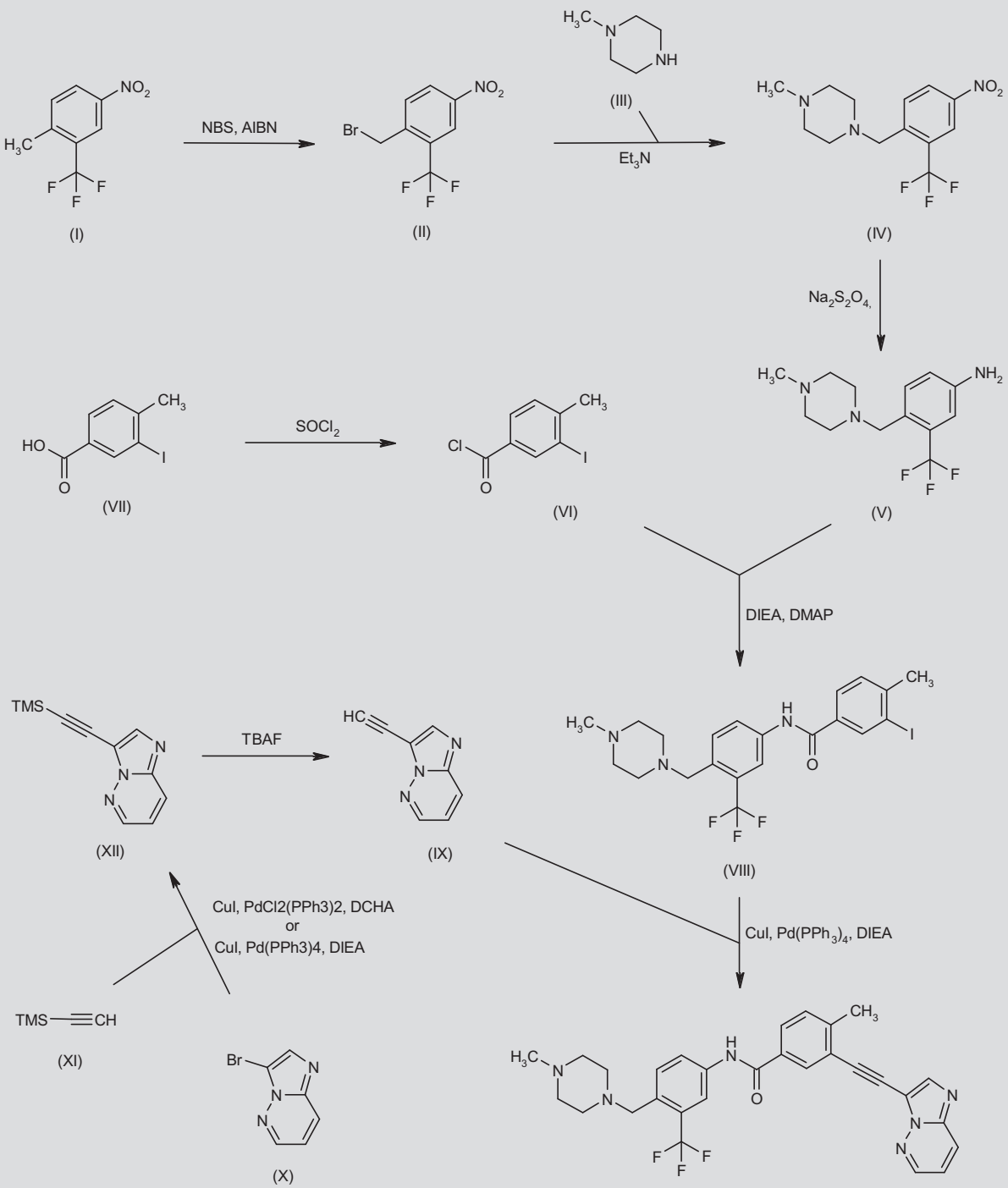
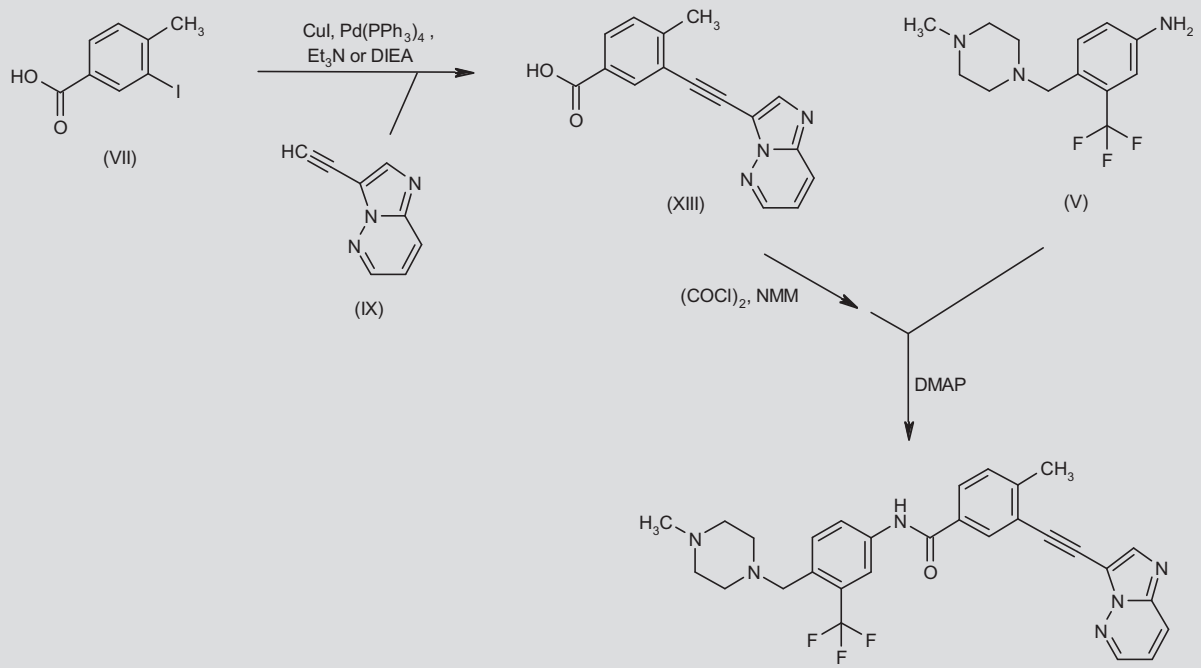
*Synthesis prepared by J. Bolòs, C. Estivill, R. Castañer. Thomson Reuters, Provença 398, 08025 Barcelona, Spain.

Scheme 1. Synthesis of Ponatinib



Scheme 2. Synthesis of Ponatinib



BACKGROUND

Chronic myeloid leukemia (CML) was estimated to affect almost 5,000 men and women in 2010, causing the death of 440 (3). This disease is characterized by the translocation of the ABL region on chromosome 9 to and fusion with the BCR region on chromosome 22. This results in the activation of the tyrosine kinase that perpetuates the pathogenetic features of CML. Early treatment modalities centered solely on the control of leukocytosis with busulfan and hydroxyurea (4). Subsequent clinical trials identified interferon alfa (rINF-α) as a treatment that provided, for the first time, cytogenetic responses, as well as improved overall survival (5).

The treatment of CML has since been revolutionized by the advent of tyrosine kinase inhibitors (TKIs) that inhibit BCR-ABL. Currently available TKIs include imatinib, dasatinib and nilotinib. In the landmark study by Kantarjian et al., imatinib was studied in late chronic-phase CML (CML-CP) after interferon failure (6). In this study, imatinib showed highly promising results, with complete hematological responses occurring in 95% of patients and major cytogenetic responses occurring in 60% of patients. Shortly after this publication, the IRIS trial compared imatinib to rINF-α with low-dose cytarabine for front-line therapy in CML-CP. This trial demonstrated superior results for imatinib with regard to hematological and cytogenetic responses, as well as tolerability (7). Additional studies have confirmed imatinib's role in the treatment of CML in the accelerated and blast phases (8, 9). Because a subset of patients were intolerant to imatinib or developed resistance, the second-generation TKIs dasatinib and nilotinib have been developed. Dasatinib and nilotinib are also highly effective in producing hematological, cytogenetic and molecular responses as second-line agents for CML-CP. More recent evidence supports their use as first-line therapy for patients in the chronic phase, as well as second-line therapy in the accelerated and blast phases of this disease (10-16). Despite these response rates, persistent intrinsic or acquired resistance to these agents is an increasing clinical problem in the treatment of all phases of this disease. While over 100 single point mutations have been documented (17), the most clinically significant is the T315I mutation, which confers resistance to all available TKIs. The new-generation TKI, ponatinib, represents an important advance in the treatment of CML because it acts as a potent inhibitor of the *BCR-ABL*^{T315I} mutation, as well as a multitude of other mutations that confer resistance to other currently available TKIs. Herein, we will review the available literature regarding ponatinib and its use in CML patients harboring the T315I mutation.

PRECLINICAL PHARMACOLOGY

Ponatinib is available as the hydrochloride salt form for oral administration and is currently available for study purposes as round,

white, film-coated tablets of 15 and 45 mg. Preclinical and clinical studies involving ponatinib suggest that the impact of this agent could extend beyond resistant CML to include *FLT3* mutated acute myeloid leukemia (AML) and Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph^+ ALL). In vitro data presented at the American Society of Hematology (ASH) meeting in 2008 by O'Hare et al. suggested that ponatinib was a potent inhibitor of all tested *BCR-ABL* mutants, including the T315I mutation (18). Various authors have previously described the concentrations required to inhibit by 50% (IC_{50}) various *BCR-ABL* mutations by the currently available TKIs, as well as ponatinib; these are reviewed in Table I (19, 20). Ponatinib also inhibits members of the proto-oncogene tyrosine-protein kinase Src, vascular endothelial growth factor receptor (VEGFR), fibroblast growth factor receptor (FGFR) and platelet-derived growth factor receptor (PDGF-R) families (19). The IC_{50} values for ponatinib acting on these kinases are listed in Table II.

A preclinical study has also been conducted in AML cell lines that expressed the *FLT3* mutation (21). This mutation is seen in approximately 20-30% of AML cases and portends a poor prognosis with inferior response rates compared to their non-mutated counterparts. The objective of this study was to evaluate the cellular activity of ponatinib in hematological malignancies that extended beyond CML. Ponatinib was shown to inhibit the phosphorylation of the target cell line (*FLT3/ITD*) at 2 nM but not in cell lines with unmutated *FLT3*. Subsequent in vivo data from a mouse xenograft model showed statistically significant inhibition of tumor growth at lower doses (1 mg/kg/day) and tumor regression at doses of 5 or 10 mg/kg/day.

PHARMACOKINETICS AND METABOLISM

Complete pharmacokinetic data in humans have not been published, although studies in animals suggest that ponatinib has an adequate oral absorption and a $t_{1/2}$ of approximately 11 hours. Additionally, as seen in these mouse models, the drug exhibits a linear relationship between the dose administered and the C_{max}. Of particular interest, ponatinib also exhibited the ability to cross the blood–brain barrier. To date, the only other available TKI shown to do so is dasatinib, although this reaches lower cerebrospinal fluid to blood ratios as compared to ponatinib (0.05-0.28 versus 1.6, respectively) (1). These data suggest that ponatinib may improve outcomes in Ph^+ diseases that affect the CNS. Preliminary data from humans suggest that the half-life of the drug is 19-45 hours and doses equal to or exceeding 15 mg daily achieved plasma concentrations of at least 40 nM, the concentration needed to inhibit cells harboring mutated *BCR-ABL* clones (22).

Table II. *Ponatinib kinase panel screening.*

Kinase	Ponatinib IC_{50} (nM)
VEGFR-1	3.7
VEGFR-2	1.5
VEGFR-3	2.3
FGFR-1	2.23
FGFR-2	1.6
FGFR-3	18.2
FGFR-4	7.7
PDGF-R-α	1.1
PDGF-R-β	7.7

CLINICAL STUDIES

Initial data on the use of ponatinib in the chronic, accelerated and blast phases of CML have been released in abstract form at several notable national meetings. In 2010, Cortes et al. presented results from an open-label phase I trial involving AP-24534 at ASH (22). The primary objective was to assess the safety of ponatinib, as well as define the maximum tolerated dose and optimal schedule, and investigate its antileukemic activity. This study included 74 patients,

Table I. *Ponatinib IC_{50} values in Ba/F3 cellular proliferation assays..*

	Ba/F3 cell proliferation assay, IC_{50} (nM)			
BCR-ABL	Ponatinib	Imatinib	Nilotinib	Dasatinib
Native	0.5	260	13	0.8
G250E	4.1	1350	48	1.8
Q252H	2.2	1325	70	3.4
Y253F	2.8	3475	125	1.4
Y253H	6.2	> 6400	450	1.3
E255K	14	5200	200	5.6
E255V	36	> 6400	430	11
T315A	1.6	971	61	125
T315I	11	> 6400	> 2000	> 1000
F317L	1.1	1050	50	7.4
F317V	10	350	nd	53
M351T	1.1	880	15	1.1
F359V	10	1825	175	2.2
H396P	1.1	850	41	0.6
M244V	2.2	2000	38	1.3
Parental	1713	> 6400	> 2000	> 200

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60 of whom had a diagnosis of CML (44 in the chronic phase, 7 in the accelerated phase and 9 in the blast phase). The remaining 14 patients had a diagnosis of Ph^+ ALL (n = 4), *FLT3*-positive AML (n = 6) and other hematological diseases (n = 4). About 95% of the CML patients had been unsuccessfully treated with two or more TKIs, and 65% had been unsuccessfully treated with three or more TKIs. Eighteen patients with *BCR-ABL*-positive disease harbored the T315I mutation at the time of study entry. During the dose-escalation portion of the trial, dose-limiting toxicities (DLTs) were documented in patients receiving 45- and 60-mg tablets and capsules. These toxicities included rash, pancreatitis, fatigue and increased alanine transaminase. Pancreatitis was the DLT at 60 mg/day and 45 mg/day was selected as the recommended dose for further testing. The incidence of grade 3 or 4 toxicity is listed in Table III. Additionally, in a previously presented abstract, two patients in a lower dose cohort (2 and 4 mg) were reported to have QT_c prolongation (23). Other toxicities associated with treatment were similar to those seen in initial reports and included fatigue, constipation, rash, headache, arthralgia, nausea, abdominal pain, fever, muscle spasms and vomiting. The response rates in CML patients are available in Table IV. Despite being heavily pretreated, 93% of patients with CML-CP had a complete hematological response (CHR), 64% had a major cytogenetic response (MCyR) and 57% had a complete cytogenetic response (CCyR). In CML-CP patients harboring the T315I mutation who received 30 mg or more of ponatinib (n = 6), all had a CHR and an MCyR and 89% had a CCyR. The response rates, as expected, were considerably lower in those patients with advanced phases of the disease (AP/BP); of the 9 patients, only 3 had an MHR and 2 had MCyR (22).

Table III. *Grade 3 and 4 toxicity seen in phase I/II studies of ponatinib.*

Grade 3 or 4 non-hematological toxicity	Grade 3 or 4 hematological toxicity
- Rash	- Thrombocytopenia
- Arthralgia	- Neutropenia
- Abdominal pain	- Anemia
- Fever	
- Pancreatitis	
- Lipase increase	

Table IV. *Treatment response in patients receiving 30 mg or more of ponatinib.*

Best response	All patients (n = 28)	T315I mutation (n = 6)
Chronic phase		
CHR	26 (93%)	6 (100%)
MCyR	18 (64%)	6 (100%)
CCyR	16 (57%)	6 (100%)
Advanced phases		
	(n = 17)	(n = 5)
CHR	6 (35%)	1 (20%)
MCyR	4 (24%)	1 (20%)
CCyR	2 (12%)	0 (0%)

CHR, complete hematological response; MCyR, major cytogenetic response; CCyR, complete cytogenetic response.

The PACE (Ponatinib Ph+ ALL and CML Evaluation) Trial is a phase II clinical trial that aims to determine the efficacy of ponatinib 45 mg orally daily in adult patients with all phases of CML, as well as Ph^+ ALL who are either resistant or intolerant to either dasatinib or nilotinib, or have the T315I mutation. Other significant inclusion criteria include an ECOG status of 0-2, "normal" renal, hepatic and pancreatic function, and a normal QT_{cF} (≤ 450 ms for males and ≤ 470 ms for females). Significant exclusion criteria for the trial include recent (≤ 7 days) treatment with any TKI, other CML-related therapy, concomitant use of immunosuppressants or any medications that are associated with torsades de pointes, significant or active cardiovascular disease, active central nervous system disease or an active or ongoing infection. Once patients are deemed eligible for the trial, patient-specific samples are tested for mutational status by direct sequencing in a central laboratory. Once the presence or absence of the T315I mutation is confirmed, patients are initiated and continued on ponatinib 45 mg/day until drug intolerance develops or disease progression occurs. The primary endpoint for this trial is the achievement of MCyR in chronic-phase patients and a major hematological response (MaHR) in accelerated- and blast-phase CML and Ph^+ ALL patients. This trial has completed patient enrollment and primary endpoint data are estimated to be completed in January of 2012 (24).

At a recent ASCO meeting, the results of 12 patients with AML included in the phase I trial were reported. The median age of AML patients was 49 (range 30-72) years. At the time of analysis (December 15, 2010), 4 (33%) AML patients remained on study (range 77-143 days) and 8 (67%) discontinued (range 10-97 days) (no discontinuations were treatment-related). Seven AML patients had documented *FLT3/ITD* at baseline, two did not carry *FLT3* alterations and three patients had inadequate samples for testing; however, these three patients had a positive *FLT3* status reported by investigative sites. Overall, seven patients with the *FLT3/ITD* mutation were FLT-3-inhibitor-naive. All patients had treatment-emergent adverse events consistent with those expected in refractory AML. Three patients had treatment-related grade 2 pancreatitis: one patient subsequently discontinued due to investigator decision; two had the event resolve and continued therapy at a reduced dose. The overall response rate in the AML cohort was 25% (3 of 12): 2 patients (29%) had a complete response with incomplete blood count recovery and 1 (14%) had a partial response. All responses observed were in the subset of patients with the *FLT3/ITD* mutation who were naive to FLT3 inhibitors: 3/7 (43%) (25).

CONCLUSIONS

To date, ponatinib is the first active agent against CML harboring the T315I mutation to be assessed in clinical trials worldwide. This provides a new and exciting therapeutic option for patients who may be intolerant of or resistant to the currently available tyrosine kinase inhibitors. Future studies may invariably examine the role of ponatinib in the frontline setting. Additionally, preliminary data suggest that ponatinib could also be effective alone or in the future in combination with traditional cytotoxic chemotherapy to improve response rates in Ph^+ ALL and AML.

SOURCE

ARIAD Pharmaceuticals, Inc. (US).

DISCLOSURES

Dr. Pinilla-Ibarz has received research support from ARIAD Pharmaceuticals. The other authors state no conflicts of interest.

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